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(6) An Improved Apparatus for Safely Feeding Fleas (Siphonaptera) in Plague Studies

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AN IMPROVED APPARATUS FOR SAFELY FEEDING FLEAS (SIPHONAPTERA) IN PLAGUE STUDIES

Abstract An apparatus incorporating improved safety features is described for feeding fleas on 3- to 8-day old suckling mice during transmission studies with pathogenic microorganisms. The safety of the entire experimental procedure is enhanced by the utilization of ice for anesthetizing fleas when examining or transferring fleas to and from the apparatus.

Several techniques and devices have been developed for feeding fleas on selected rodent species (Eskey & Haas, 1939, Public Health Rep. **54:** 1467–81, Wheeler & Douglas, 1941, Proc. Soc. Exp. Biol. Med. **47:** 65–66; Wheeler & Douglas, 1945, J. Infect. Dis. **77:** 1–12, Burroughs, 1947, J. Hyg. **45:** 369–71; Holdenried, 1952, J. Infect. Dis. **90:** 131–40, Burroughs, 1953, Parasitology **43:** 35–48; Cavanaugh, Stark, Marshall & Rust, 1972, J. Med. Entomol. **9:** 113–14). Those procedures were used to establish experimental vector efficiencies of various rodent fleas and allowed those workers to make significant contributions toward understanding the epidemiology of urban and sylvatic plague. We required more stringent safety controls than are inherent in existing systems. Planned studies with *Yersinia pestis* dictated an absolute accountability for every flea. This requirement stimulated development of an improved apparatus to maintain fleas and feed them on suckling mice[1] (FIG. 1).

The apparatus, incorporating some of the Berlese principles, is constructed of a clear, acrylic tube (outside diameter, 2.6 cm, inside diameter, 1.9 cm) divided into 3 easily connectable sections. The bottom (section 1), 3.0 cm long, is filled with substrate composed of a charcoal/plaster of paris mixture (1:9), which provides a continual source of humidity in the flea holding chamber (section 2). The substrate is moistened weekly by adding a few drops of distilled water. The smooth surface and length of section 2 (21.0 cm) make it nearly impossible for fleas

[1]In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Science, National Research Council.

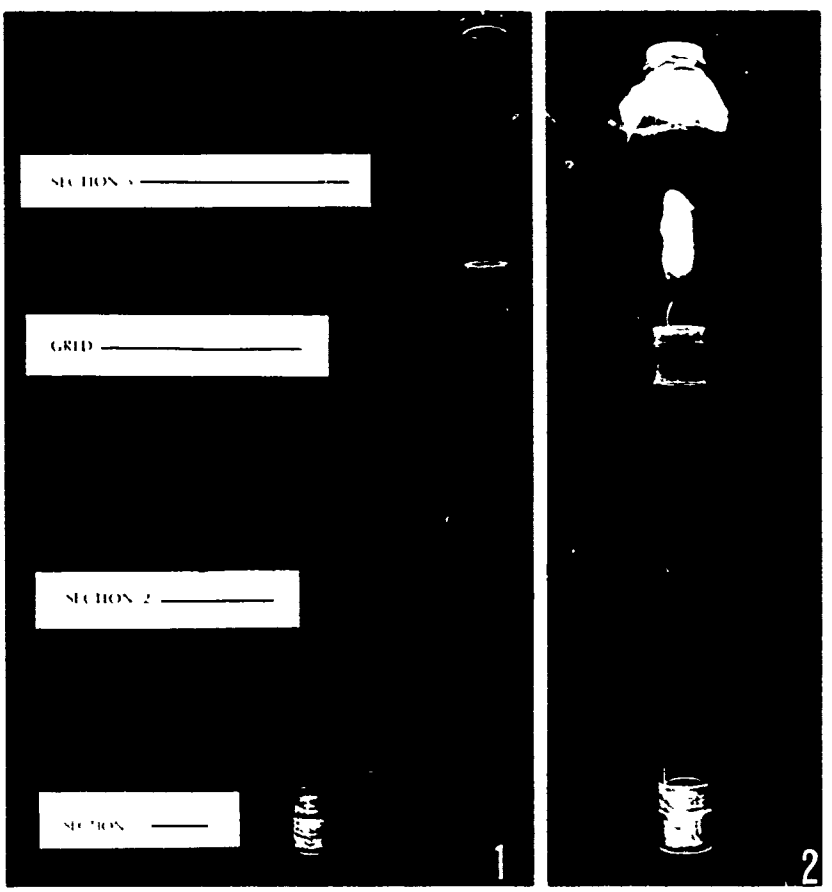

FIG. 1–2. 1, Disassembled flea feeding apparatus illustrating components. 2, Assembled flea feeding apparatus illustrating horizontal position during flea feeding.

to escape by climbing or jumping while the mouse holding chamber (section 3) is being connected or disconnected. A stainless steel grid (2.2 cm in diameter with 0.25-cm holes) separates sections 2 and 3, and confines the rodent to section 3 (15 cm long).

Fleas aspirated with a standard WHO aspirator are anesthetized with ice, and a moistened camel hair brush is used to place the desired number of fleas in sections 1 and 2. The open end of the apparatus is covered with a nylon mesh screen to prevent the fleas from escaping and is maintained in a vertical position in a support rack except while the fleas are feeding. For feeding, a 3- to 8-day-old suckling mouse is placed in section 3. The open end of section 3 is covered with nylon mesh to prevent the escape of fleas. The nylon mesh is removed from section 2 and section 2 is joined to section 3. The entire apparatus is inverted, allowing the fleas to fall into section 3. The apparatus is placed in a horizontal position while the fleas feed (FIG. 2). When feeding is complete, the apparatus is placed upright with section 1 and 2 down until all fleas have returned to section 2. This is easily determined by visual inspection. The clear acrylic composition facilitates counting the limited number of fleas being tested. Section 3 is removed, and section 2 is covered with nylon mesh. Fleas never leave section 2 except while feeding or when they are anesthetized and removed for microscopic examination. Carbon dioxide may be used to anesthetize fleas; however, a preferable method includes emptying the fleas from sections 1 and 2 directly onto the wet surface of a shallow pan of solid ice. The fleas are instantaneously anesthetized, can be manipulated under a dissecting microscope while on the surface of the ice, and recover rapidly when returned to sections 1 and 2. The apparatus provides a safe and completely closed system except during connection and disconnection of sections 2 and 3. As a further precaution all manipulations are performed and fleas are stored in a 60 × 60 × 18 cm high stainless steel pan.

There are 2 problems inherent in the apparatus. First, accumulation of condensation on the inside of section 2 results in flea mortality. The condensation can be removed by transferring fleas to section 3 as previously described, disconnecting sections 2 and 3, covering the proximal end of section 3 with nylon mesh, and passing a cotton gauze pad through section 2. After condensation is removed, the fleas may be transferred back to section 2, which is re-covered with nylon mesh. Second, autoclaving distorts and discolors acrylic plastic, but the simplicity of the design facilitates cold sterilization with any of the usual germicidal agents except alcohols.

The apparatus provides a simple and reliable method of maintaining and feeding adult fleas for *Y. pestis* studies and other flea-borne pathogens, i.e., *Rickettsia typhi*.

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